Bioorganic & Medicinal Chemistry Letters 28 (2018) 2068–2073

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Harnessing biosynthesis and quantitative NMR for late stage functionalization of lead molecules: Application to the M1 positive allosteric modulator (PAM) program

Michael A. Brodney [a,*], Raman Sharma [b], John T. Lazzaro [b], Gregory S. Walker [b], R. Scott Obach [b]

[a] Pfizer Worldwide Research and Development, 610 Main Street, Cambridge, MA 02139, United States
[b] Pfizer Worldwide Research and Development, Eastern Point Road, Groton, CT 06340, United States

ARTICLE INFO

Article history:
Received 3 March 2018
Revised 15 April 2018
Accepted 22 April 2018
Available online 23 April 2018

Keywords:
Late-stage diversification
Biosynthesis
M1 positive allosteric modulator (PAM) program
Medicinal chemistry design cycle

ABSTRACT

A facile method for late stage diversification of lead molecules for the M1 PAM program using biosynthesis is described. Liver microsomes from several species are screened to identify a high turnover system. Subsequent incubations using less than 1 mg of substrate generate nanomole quantities of drug metabolites that are purified, characterized by microcryoprobe NMR spectroscopy, and quantified to known concentrations to enable rapid biology testing. The late-stage diversification of lead compounds provides rapid SAR feedback to the medicinal chemistry design cycle.

Late-stage diversification (LSD) of lead molecules has gained considerable attention in drug discovery as a method to accelerate analog generation and reduce costs.[1] A number of chemical methods have been used in LSD such as rhodium or iridium catalyzed C—H insertion or oxidative functionalization using iron catalysis.[2–4] Recently, an alternative oxidative functionalization method that involves biocatalysis with recombinant cytochrome P450 enzymes has been reported.[5–7] Our method was originally developed as a means to identify and characterize drug metabolites. A key aspect to the method is the use of quantitative NMR (qNMR) spectroscopy which uses a mathematically inserted internal standard allowing for the quantification and subsequent characterization of drug samples (see workflow, Fig. 1). Using less than 1 mg of substrate, a number of diverse products can be generated. Initially, the metabolism of the selected substrates was examined in liver microsomes from several different species (i.e. mouse, rat, gerbil, hamster, guinea pig, rabbit, dog, monkey, and human). The metabolite profile was evaluated by UHPLC-UV-HRMS analysis indicating the quantitative prevalence of individual metabolites and information on the type of metabolite generated (e.g. hydroxylation, heteroatom dealkylation, dehydrogenation, etc). The specific microsomal system offering the best consumption of the substrate and a variety of products arising from one or two transformations were selected for the subsequent biosynthesis. Creation of very small quantities of such products (~5–200 nmoles each), quantified in d_6-DMSO solutions (final concentrations typically ranging from ~0.1 to 5 mM), permits these materials to be tested for primary bioactivity and examined in additional *in vitro* assays.

To establish the value of this approach in drug discovery, we recently described LSD for the design of phosphodiesterase 2 (PDE2) inhibitors which generated novel analogs with reduced clearance and risk of drug-drug interactions.[8] By coupling biosynthesis with a subsequent chemical modification, an even greater diversity of analogs and SAR using LSD is possible. As an example, several known drugs were subjected to this LSD method followed by a simple deoxyfluorination procedure yielding an overall C—H to C—F transformation.[9]

The muscarinic acetylcholine (mAchR) receptors M_1–M_5 are a family of five G protein-coupled receptors that continue to be attractive targets for drug development. Decades of preclinical and clinical data on the pharmacology of the muscarinic receptors have made them an attractive target in neurology and psychiatry for addressing the cognitive and negative symptoms associated with schizophrenia and Alzheimer's disease.[10–12] Selective activation of M_1 with a positive allosteric modulator (PAM) has emerged as a new approach to achieve selective M_1 activation.[13] We recently disclosed several series of M_1 PAMs including pyridine

* Corresponding author.
E-mail address: michael.a.brodney@hotmail.com (M.A. Brodney).

https://doi.org/10.1016/j.bmcl.2018.04.054
0960-894X/

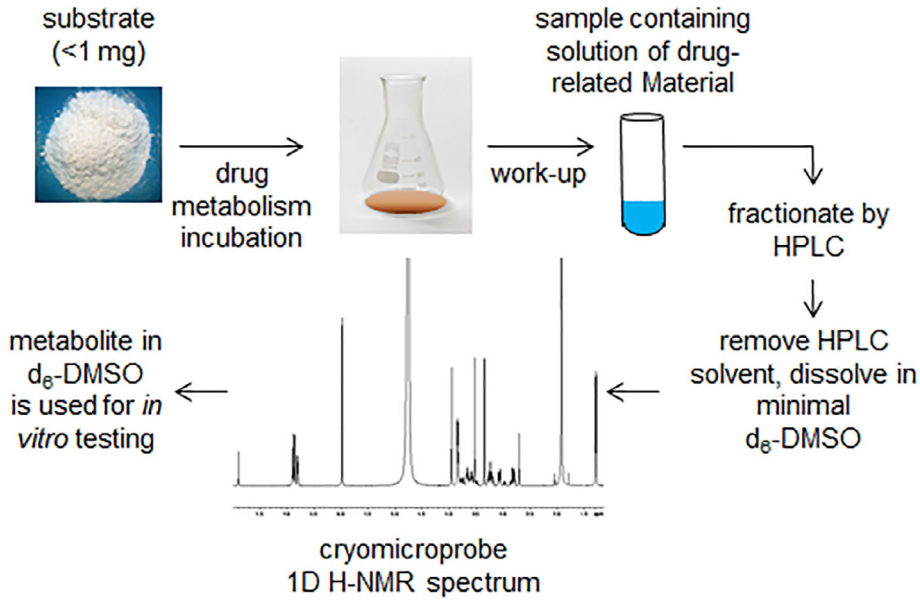

Fig. 1. Workflow of late-stage lead diversification microsomal screen.

amides (**1**–**2**),[14] lactams (**3**),[15] and azaindoles (**4**–**6**)[16] that display excellent potency, selectivity, CNS penetration, and efficacy in several *in vivo* models (Fig. 2). Our goal was to apply LSD to a range of analogs in the M1 PAM program with the goal of expanding SAR, reducing design-synthesis cycles, and cutting synthetic costs per analog generated.

As shown in Scheme 1, compound **1** displayed favorable activation potency (M1 PAM EC_{50} = 109 nM), high metabolic stability in human liver microsomes (HLM $CL_{int,s}$ < 8 mL/min/kg), low P-gp mediated efflux (MDR Er = 1.4) and high passive permeability (RRCK = 21 × 10^{-6} cm/s). Based on the promising overall profile and desire to further understand the SAR, compound **1** was subjected to metabolism by a panel of liver microsomal samples from several species, including human, with the goal of identifying a species with high substrate turnover leading to an array of products. To this end, the metabolite profiles were analyzed and compared by HPLC-MS revealing that rabbit liver microsomes offered the best promise of delivering a variety of new products. A rabbit liver microsomal incubation was therefore carried out at a scale of 600 nmol (0.24 mg) of substrate in an incubation volume of 30 mL for 1 h. From this single incubation, six unique and diverse analogs **7**–12 were isolated, characterized, and tested for M1 activation potency (Scheme 1). The oxidative products included hydroxylation on the 5-methyl group of the pyridine (**7**), the 4-position of the pyrazole ring (**8**), the 2 and 6-position of the THP ring (**9** and **10**), the benzylic position (**11**), and finally a product resulting from ring opening of the THP ring (**12**). Oxidative product **7** was only 3-fold less active than the parent **1**, suggested polarity was well tolerated in this position. Unfortunately, the additional hydrogen bond resulted in a significant increase in P-gp mediated efflux (MDR Er = 15.9). However, the primary alcohol represented a novel template for further SAR exploration as this group could be used as a precursor to further chemical elaboration. Oxidation on the 4-position of the pyrazole ring resulted in compound **8** that exhibited improved potency and lower lipophilicity relative to the parent **1**. This SAR point in particular suggested that further exploration of the heteroaromatic ring may be a strategy to modify physicochemical properties. Hydroxylation on the THP ring (**9**–**10**) or products derived from oxidation and ring opening (**12**) were less active than parent. Despite the lack of activity, these were important SAR points due to the synthetic complexity that would have required for the resulting products. Finally, oxidation on the benzylic position generated diastereomers (**11a** and **11b**) in which neither was active. This result supported a critical SAR observation regarding the active confirmation required for activity. The absolute configurations of **9**, **10**, **11a**, and **11b** were not determined at this time due to the unfavorable SAR but efforts to establish this information can be pursued when relative or absolute stereochemistry is considered valuable information for the SAR of the chemical series. Overall, the rapid diversification of compound **1** highlighted a key efficiency of this LSD methodology as the targeted synthesis of compounds **7**–**12** would be lengthy and time-consuming.

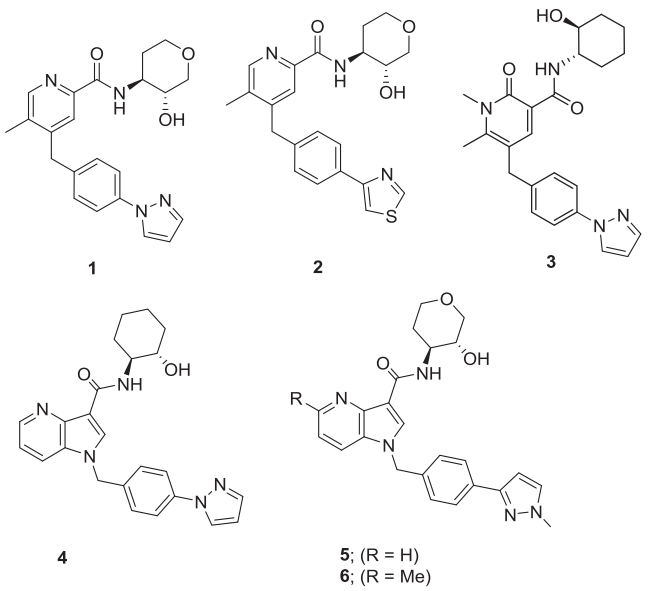

Fig. 2. Representative M1 PAM Chemical Matter.

We next selected the closely related molecule **2** to profile via the LSD method with the goal of understanding the translatability of the SAR across related molecules in a series (Scheme 2). The initial selection of microsomes with high-turnover was met with limited success due to the high metabolic stability of **2**. Ultimately,